ION DISTRIBUTION AND TRANSPORT IN THE RED MARINE ALGA, GRACILARIA FOLIIFERA [1, 2]

JOHN GUTKNECHT

Bureau of Commercial Fisheries, Radiobiological Laboratory, Beaufort, N. C. 28516

Marine algae were the object of many early studies of electrolyte permeability and transport (see Blinks, 1940; Osterhout, 1940). However, there have been few recent investigations of ionic relations in marine algae. In *Ulva* and *Hormosira* independent and oppositely directly Na^+ and K^+ transport systems were proposed (Scott and Hayward, 1954, 1955; Bergquist, 1958, respectively). *Porphyra,* a non-vacuolated red alga, actively extruded Na^+ and passively accumulated K^+ (Eppley, 1958a, 1958b, 1959, personal communication). MacRobbie and Dainty (1958a, 1958b) demonstrated active efflux of Na^+ and active influx of Cl^- in *Rhodymenia,* a vacuolated red alga, and in *Nitellopsis,* a brackish water characean. *Halicystis* actively extruded Na^+ and actively absorbed Cl^-, whereas K^+ was in electrochemical equilibrium between vacuole and sea water (Blount and Levedahl, 1960).

In some, but not all, of these studies membrane potential measurements were made in conjunction with ion distribution and flux measurements. As pointed out by Ussing (1960) and Dainty (1962), this information is needed to determine exactly which ions are actively transported, *i.e.,* moved against an electrochemical potential gradient.

This is a study of alkali and halide ion transport in *Gracilaria foliifera,* a red marine alga. The major monovalent ions, Na^+, K^+ and Cl^-, are included, as well as the less abundant ions, Rb^+, Cs^+, Br^- and I^-. The latter were included for comparative purposes and also because of their radiobiological importance as components of fallout and nuclear reactor wastes. A primary objective of this work was to determine which of these ions are actively transported by cells of *Gracilaria.* The major criterion for active transport was the existence of an appreciable difference between the calculated Nernst equilibrium potential for each ion and the measured vacuole potential. The effects of some environmental factors, mainly light and anaerobiosis, on ion fluxes and their rate coefficients were studied also.

Gracilaria foliifera is a red seaweed common on the North Carolina Coast. Its slightly compressed branches are composed of large vacuolated cells, 100–150 μ in diameter, surrounded by several layers of small cells, 10–20 μ in diameter, and a tough outer cuticle. Visual estimates indicate that the vacuoles of the large cells comprise more than half the tissue volume. Most of the remaining volume, as shown below, is extracellular space and some impermeable material. The protoplasm comprises only a small fraction of the tissue.

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Methods

Experimental procedure

Gracilaria was collected from rock jetties near the Beaufort harbor inlet and placed in 40-liter plastic tanks containing aerated sea water. Constant illumination was provided by two 40-watt "daylight" fluorescent tubes placed about 60 cm. from the seaweed. Water temperature ranged from 15° to 20° C. The alga was kept under these conditions for several months without apparent deterioration.

Experiments were conducted with small branches of the seaweed, 3–4 cm. and 10–30 mg. each, contained in 2-liter flasks of aerated medium. The basic medium was natural sea water (salinity 32–35‰), buffered at pH 7.8–7.9 with tris(hydroxymethyl)aminomethane (10 m*M*) and containing added $NaNO_3$ (100 μM) and NaH_2PO_4 (50 μM). The test solutions were changed daily. Experiments were conducted either in the dark (flasks painted black) or under constant illumination of 8500 ± 1000 lux provided by six 40-watt "daylight" tubes, 20–30 cm. from the flasks. The experimental temperature was 24 ± 1° C. Under these conditions the growth rate in the light was 0.5–1.0%/hr., whereas no detectable growth occurred in the dark.

Sodium and potassium were measured by flame photometry (Beckman DU spectrophotometer with flame attachment),[3] and chloride was measured with an Aminco-Cotlove automatic chloride titrator. Sodium and potassium were extracted by soaking tissues overnight in HNO_3 (1.0 or 0.2 N). Chloride was extracted either by this method or with hot distilled water. Similar results were obtained with both methods.

Cesium, Rb^+ and I were not measured chemically. The relative intracellular concentration of these elements was determined by radioactivity measurements, and is expressed as a concentration ratio (c.p.m./g. cell water) : (c.p.m./ml. environment). The natural concentrations of Cs^+ and Rb^+ in sea water are about 0.04 μeq./l. and 1.4 μeq./l., respectively (Smales and Salmon, 1955). The total iodine concentration in sea water is about 0.4 μeq./l. (Barkely and Thompson, 1960; Sugawara *et al.*, 1962).

Gamma-emitting radioisotopes (Cs^{137}, Rb^{86}, K^{42}, Br^{82} and Na^{22}) were measured in a 3-inch well-type scintillation detector connected to a single-channel analyzer (Baird-Atomic Model 510). Tissues were removed periodically from the flasks, blotted with absorbent tissue, wrapped in plastic, counted, weighed and returned to their solutions. Carbon-14 and Cl^{36} were measured in an end-window gas flow detector with a low-background attachment (Nuclear Chicago Model C-115). Several 1-ml. aliquots of neutralized tissue extract or medium were dried in planchets for counting.

Radioisotopes were purchased from the Oak Ridge National Laboratory, Oak Ridge, Tenn. (K^{42}, Rb^{86}, Cs^{137}, Cl^{36}, Br^{82} and I^{131}); Nuclear Science and Engineering, Pittsburgh, Pa. (Na^{22}); and Calbiochem, Los Angeles, Cal. (C^{14}-mannitol and C^{14}-sucrose). Tracers were either "carrier-free" or of sufficiently high specific activity so that the natural concentration of the element was increased less than 3%.

The electrical potential of the vacuole was measured with glass microcapillary electrodes and a high-impedance voltmeter (Keithley Model 600A). Pipettes with tip diameters of 4–8 μ were drawn from 1 mm. Pyrex tubing and filled with

[3] Trade names referred to in this publication do not imply endorsement.

0.5 *M* KCl by boiling under reduced pressure. The pipette was connected to the voltmeter by a KCl-agar bridge and calomel half-cell. An identical half-cell and sea-water-agar bridge completed the circuit, making contact with the bathing sea water which was taken as ground. The electrodes had tip potentials of less than 2 mv. in either sea water or 0.5 *M* KCl.

To measure the vacuole potential, a branch was held rigidly with waterproof putty in a glass dish of sea water under a dissecting microscope. The cells were exposed by an oblique cross-sectional cut, and the puncture was made with the aid of a micromanipulator. Ten to 20 minutes were usually required for the potential to reach a stable value. After this, only potentials which were stable (±1 mv.) for at least 30 minutes were recorded.

Estimation of ion fluxes and rate coefficients

The difficulties of estimating ion fluxes and their rate constants in a heterogeneous cell population were emphasized by MacRobbie and Dainty (1958a) in their study of Na^+ and K^+ fluxes in the seaweed *Rhodymenia*. Since their admonitions apply also to the tissues of *Gracilaria*, fluxes were estimated in terms of cell water rather than in terms of membrane area. Also, rate constants, or rate coefficients (since they varied with time), were measured during successive time intervals and averaged.

Unidirectional fluxes were estimated for the ions in which specific activities were known (Na^+, K^+ and Cl^-), using the relations

$$M_i = \frac{C_i^*}{t}\frac{C_o}{C_o^*}$$

$$M_o = k_o C_i$$

where M_i and M_o are the initial influx and efflux in meq. (kg. cell water)$^{-1}$ hours^{-1}; C_o^* and C_o are the labeled ion and total ion concentrations outside; C_i is the total intracellular ion concentration; C_i^* is the labeled ion concentration inside the cells at time, t; and k_o is the rate of coefficient for efflux, calculated as shown below. Influx values were calculated by using data from the early portion of isotope uptake experiments, when the efflux of labeled ions was considered negligible.

Rate coefficients for isotope influx (k_i) and efflux (k_o) were calculated from the equations

$$k_i = \frac{2.3}{t}\log\frac{C_{i,oo}^*}{C_{i,oo}^* - C_i^*}$$

$$k_o = \frac{2.3}{t}\log\frac{C_{i,0}^*}{C_i^*}$$

where $C_{i,oo}^*$ is the intracellular labeled ion concentration at isotopic equilibrium; $C_{i,0}^*$ is the initial intracellular labeled ion concentration; and C_i^* and t have the same meanings as before. The two rate coefficients, one calculated from isotope influx data and the other from isotope efflux data, provided a means of comparing the unidirectional fluxes of ions for which specific activities were not known (Cs^+, Rb^+, Br^- and I^-).

These equations are similar to those used recently by House (1963). They are derived from standard flux equations, treating the cells as a single compartment (see, for example, Sheppard, 1962). In experiments involving tracer efflux into a large nonlabeled environment, the usual plot of log activity remaining *vs.* time was often nearly linear, indicating fairly good agreement with predicted single-compartment behavior.

Results

Tissue water, extracellular space and cell water

To estimate intracellular ion concentrations, the amounts of tissue water and extracellular space must be known. Tissue water, measured by comparing the fresh weight (after blotting with absorbent tissue) with the dry weight (24 hr. at 105° C.), was found to be $80 \pm 1\%$ (6). (Results are quoted in the sequence: mean, standard error and [in parentheses] number of measurements.)

Extracellular space (mainly the free space in the cell walls and between the cells) was measured by soaking tissues for 2–3 hours in sea water containing C^{14}-mannitol or C^{14}-sucrose. Tissues were then blotted, weighed, and washed in 50 ml. unlabeled sea water. From the amount of C^{14} which diffused from the tissues to the environment (loss was complete after 15–30 minutes), it was calculated that the tissue contained $19 \pm 2\%$ (5) sucrose or mannitol space. The same method applied to tissues freshly killed (by immersing for 10 seconds in sea water at 90° C.) gave a value of $91 \pm 2\%$ free space, suggesting that about 9% of the tissue was impermeable to the labeled sugar. Although the agreement may be fortuitous, the latter value is close to the 11% dry weight found above.

Extracellular space also was checked by using Na^{22}, Cl^{36} and Br^{82}. The amounts of these ions washed out in 8–10 minutes with either 0.6 *M* sucrose or unlabeled sea water agreed with the amounts predicted on the basis of 19% sucrose or mannitol space. This supports the assumptions that ion concentrations in the extracellular space are similar to those in sea water and that exchange of intracellular ions during a 10-minute wash in sucrose or sea water is insignificant. The other ions studied (Rb^+, Cs^+, K^+ and I^-) were predominantly intracellular, so that some extracellular adsorption (although there was little indication of this) would not appreciably affect the estimated intracellular ion concentrations.

Cell water was estimated, using the above figures, to comprise 69–71% of the tissue (*i.e.*, the difference between per cent tissue water and per cent extracellular space). Therefore, the intracellular ion concentrations were calculated as $100/70 = 1.4$ times the tissue ion concentration, either following a 10-minute wash in 0.6 *M* sucrose or after subtracting the estimated concentration of ions in the extracellular space.

Electrical potential of the vacuole

The vacuole potential, measured by means of microcapillary electrodes, was -81 ± 2 mv. (12). The highest recorded potential was -92 mv. The measurements were usually made after 12–48 hours of illumination, although several hours of darkness had no obvious effect on the potential.

Ion distribution and equilibrium potentials

The concentrations of monovalent ions in *Gracilaria* and in sea water are shown in Table I. Concentration ratios (C_i/C_o) are based either on chemical and radioactivity measurements (Na^+, K^+ and Cl^-) or on radioactivity measurements alone (Rb^+, Cs^+, Br^- and I^-). The C_i/C_o values obtained in tracer experiments represent the steady-state levels attained in the light (after 10 hours to 9 days exposure, depending upon the ion). Chemical measurements of Na^+, K^+ and Cl^- were made after about 48 hours illumination.

Potassium, the principal intracellular cation in *Gracilaria,* was concentrated about 60-fold (Table I). Sodium, in contrast, was excluded by a factor of about seven. Chloride, the principal intracellular anion, was present at over 60% of

TABLE I

Concentrations and equilibrium potentials of monovalent ions in Gracilaria and in sea water, ***

Ion	Sea water (meq./l.) (C_o)	*Gracilaria* (meq./kg. fresh wt.)	*Gracilaria* (meq./kg. cell H_2O) (C_i)	Concentration ratio (C_i/C_o)	Equilibrium potential (mv.) (E_j)
Na^+	471 ± 11 (11)	161	66.3 ± 4.6 (13)	0.14 ± 0.01 (11)	+50
K^+	11.0 ± 0.2 (9)	488	680 ± 12 (12)	61.8 ± 1.1 (9)	−105
Rb^+	1.4×10^{-3}	—	—	148 ± 5 (19)	−127
Cs^+	0.04×10^{-3}	—	—	28.6 ± 2.5 (8)	−85
Cl^-	532 ± 3 (8)	436	462 ± 17 (8)	0.87 ± 0.03 (8)	−3.6
Br^-	0.81	—	—	0.57 ± 0.03 (4)	−14
I^-	0.4×10^{-3}	—	—	150 ± 7 (4)	+128

* Sea water concentrations of Rb^+, Cs^+, Br^-, and I^- are approximate, based on published values. Concentration ratios for these ions, based on radioactivity measurements, are more accurate, however.

** Results expressed as mean, S.E. and (in parentheses) number of measurements.

the concentrations of Na^+ plus K^+. About 70% of the tissue Na^+ and 25% of the tissue Cl^- were extracellular. Rubidium, Cs^+ and I^- were all highly concentrated by cells of *Gracilaria,* although they were of little quantitative importance compared to Na^+, K^+ and Cl^-.

An equilibrium potential may be calculated for each ion, using an approximation of the Nernst equation

$$E_j = \frac{-2.3\ RT}{z_j F} \log \frac{C_i}{C_o}$$

where E_j is the electrical potential across a membrane separating an ion at two concentrations, C_i and C_o. The constants R, T, and F have their usual meanings, and z_j is the algebraic valency of the ion, j. An appreciable difference between the vacuole potential (E) and the equilibrium potential (E_j) indicates that the ion is not in passive flux equilibrium across the membrane and suggests, therefore, active transport of that ion (see Dainty, 1962).

Most of the calculated equilibrium potentials shown in Table I do not agree

TABLE II

Rate coefficients for isotope fluxes in Gracilaria. Effects of light and darkness, ***

Ion	Rate coeff. of isotope influx (k_i)	Rate coeff. of isotope efflux (k_o)	
	Light	Light	Dark
Na^+	30 ± 1 (9)	80 ± 9 (8)	14 ± 4 (6)
K^+	20 ± 2 (6)	9.8 ± 0.5 (7)	0.47 ± 0.09 (5)
Rb^+	1.6 ± 0.2 (11)	0.67 ± 0.1 (13)	0.09 ± 0.02 (4)
Cs^+	1.9 ± 0.2 (9)	0.46 ± 0.05 (14)	0.22 ± 0.08 (4)
Cl^-	1.8 ± 0.1 (9)	0.58 ± 0.1 (6)	0.14 ± 0.03 (5)
Br^-	0.81 ± 0.08 (7)	0.88 ± 0.08 (5)	0.35 ± 0.05 (5)
I^-	2.5 ± 0.3 (5)	0.55 ± 0.04 (3)	0.21 ± 0.06 (3)

* Units are $10^2 hr.^{-1}$.

** Results expressed as mean, S.E. and (in parentheses) number of measurements.

with the measured vacuole potential of −81 mv. Of the major ions, Na^+ is farthest from electrochemical equilibrium, both electrical and chemical gradients being directed inward. This suggests that Na^+ is actively transported out of the cells. Potassium, Rb^+, Cl^- and Br^- are all concentrated inside at considerably higher levels than would be predicted from the vacuole potential of −81 mv. Cesium is close to electrochemical equilibrium, and the status of I^- is uncertain since, as is shown below, there is appreciable intracellular binding of I^-. Thus K^+, Rb^+, Cl^- and Br^- appear to be actively absorbed by cells of *Gracilaria,* and Na^+ appears to be actively extruded.

To justify using the Nernst equation, major shifts in ion distribution should not occur under the experimental conditions. To check this, tissues were illuminated for 48 hours, then kept for 5 days in the dark, and also under nitrogen in the dark. Under these conditions the intracellular K^+ and Cl^- concentrations decreased less than 10%. Similarly, there was a net loss of about 5% of the radioactive Rb^+ and Cs^+ from maximally labeled tissues which were kept for 5 days under the same two conditions, in a constant radioactive environment. Intracellular Na^+ decreased in the dark from about 70 to 50 meq./kg. cell water, and increased under nitrogen from about 70 to 90 meq./kg. cell water. The net ion movements were slow compared to the unidirectional fluxes given below. Thus, under the conditions of this study, intracellular ion concentrations remained approximately constant.

TABLE III

Ion fluxes in Gracilaria. Effects of light and darkness, ***

Ion	Influx (M_i)		Efflux (M_o)	
	Light	Dark	Light	Dark
Na^+	18 ± 2 (5)	2.1 ± 0.3 (4)	54 ± 6 (8)	8.8 ± 2.5 (6)
K^+	101 ± 2 (8)	9.1 ± 3.1 (6)	66 ± 4 (7)	3.2 ± 0.5 (5)
Cl^-	2.0 ± 0.5 (6)	1.0 ± 0.4 (5)	2.7 ± 0.5 (6)	0.62 ± 0.2 (5)

* Units are meq. (kg. cell water)$^{-1}$hr.$^{-1}$.

** Results expressed as mean, S.E. and (in parentheses) number of measurements.

Another requirement for calculating equilibrium potentials is that intracellular ions be in solution rather than adsorbed or bound to cytoplasmic sites. In *Gracilaria* intracellular ion binding was expected to be small, since the cytoplasm comprises only a small fraction of the large vacuolated cells. With K^{42}, Rb^{86} and Cs^{137} this was substantiated by the observation that maximally labeled tissues, when killed and held in the same radioactive environment, lost virtually all of their radioactivity

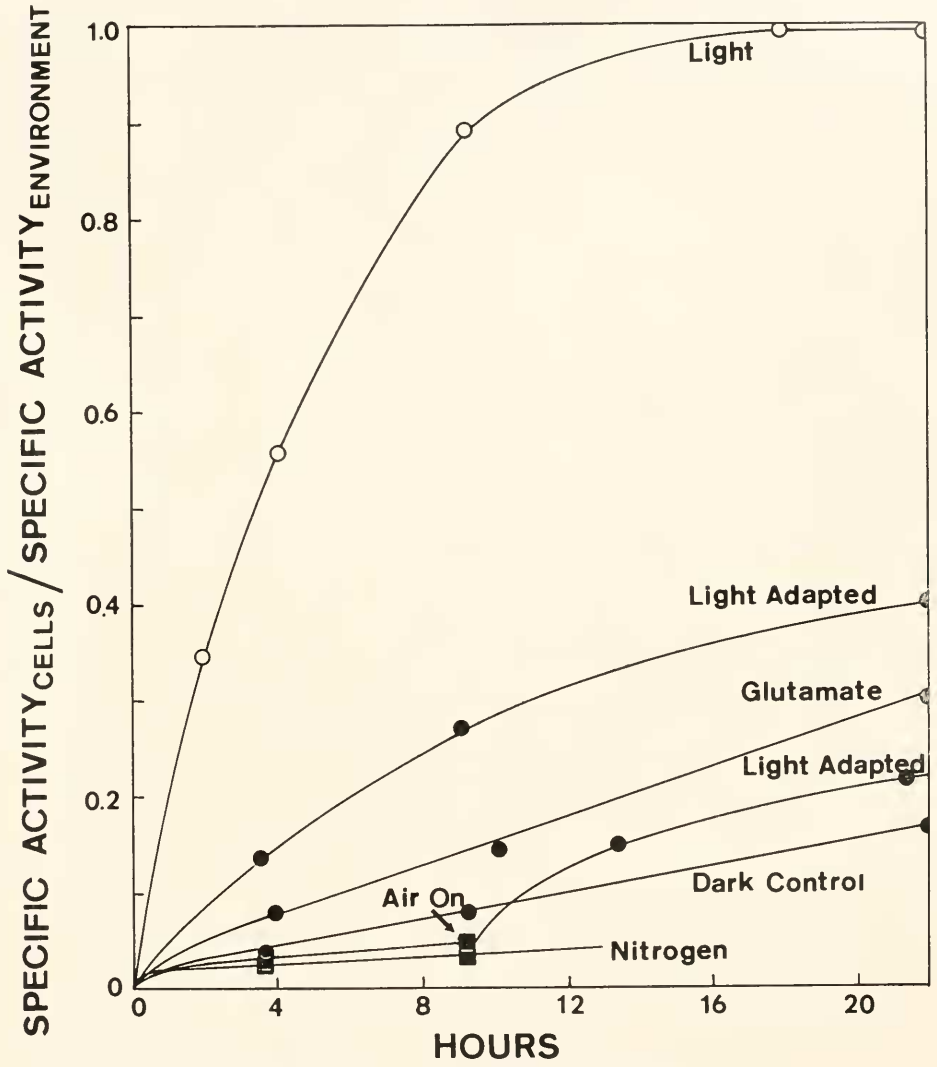

FIGURE 1. Potassium-42 uptake by cells of *Gracilaria*. Effects of light (8500 lux) (○), darkness (●), anaerobiosis (nitrogen) (■), glutamate (50 m*M*), and previous exposure to light (20 hours at 8500 lux). Arrow indicates the addition of air to tissues previously exposed to light, then placed in a K^{42} environment under nitrogen. Each point represents a measurement of 30–40 branches.

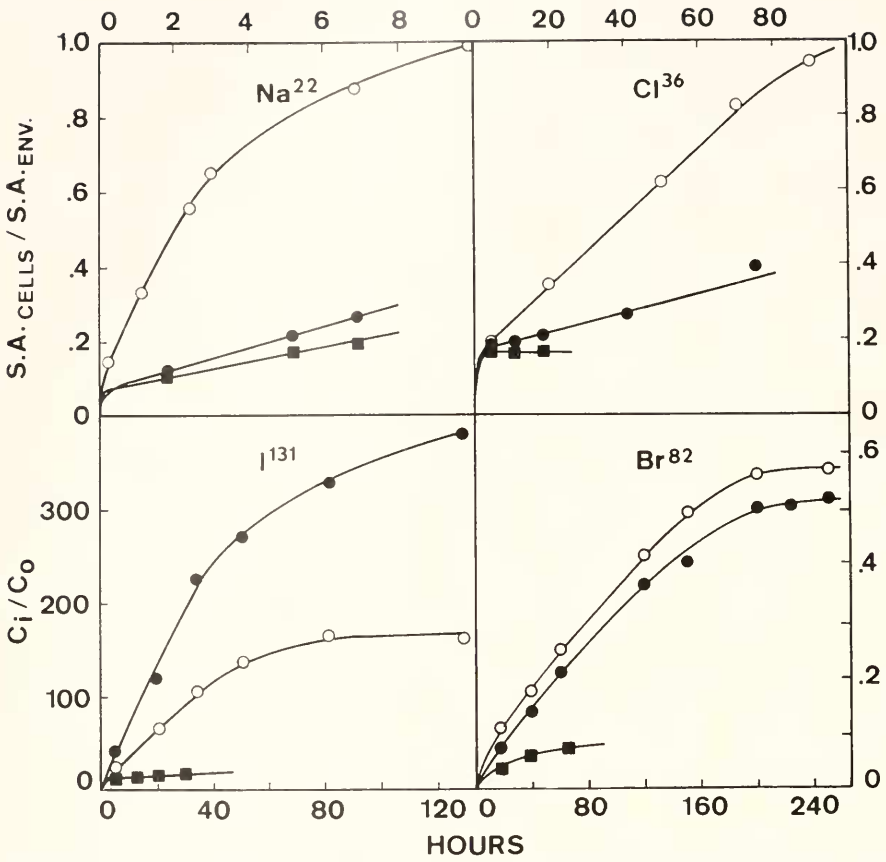

FIGURE 2. Uptake of Na^{22}, Cl^{36}, Br^{82} and I^{131} by cells of *Gracilaria*. Effects of light (○), darkness (●), and anaerobiosis (■). Radioactivity of cells expressed as a concentration ratio (C_i/C_o) or as the ratio of the specific activity (S.A.) of the cells to the S.A. of the environment. Each point represents a measurement of 15–40 branches. Note the differences in time and radioactivity scales.

in 20–30 minutes. Possible adsorption of the trace elements Rb^+ and Cs^+ was checked also by exposing the tissues to a wide range of low concentrations (0.01–100 μeq./l.). The uptake data, plotted as a Freundlich adsorption isotherm (Freundlich, 1926), gave linear log C_i *vs.* log C_o curves with a slope of 1.0 (Gutknecht, 1965). In other words, uptake over this range of concentrations was exactly proportional to external concentration, which is not likely in an adsorption-type reaction (Briggs, Hope and Robertson, 1961).

Iodide-131, in contrast to the other ions studied, was at least partly bound. Maximally labeled tissues, when killed and held in their original radioactive environment, retained about 25% of their radioactivity. Interestingly, tissues exposed to I^{131} in the dark contained a much larger amount of bound radioactivity. This difference was reflected in a higher rate of uptake as well as a higher C_i/C_o of 416 ± 20 (4), compared to the value of 150 found in the light (Table I).

All of the Na^+, K^+ and Cl^- in *Gracilaria* appeared to be exchangeable in the

light. The specific activities of the tissues and environment became equal after exposure periods of about 10 hours for Na^{22}, 20 hours for K^{42} and 100 hours for Cl^{36}. In general, all the ions except I^- appeared to be unbound and exchangeable and are no doubt largely in solution in the vacuolar sap. It is not likely, therefore, that ion binding plays an important role in determining the steady-state distribution of ions in *Gracilaria*.

Ion fluxes and rate coefficients

Ion fluxes were measured in the light (8500 lux), dark and under anaerobic conditions (continuous bubbling with commercial nitrogen in the dark). The effects of previous exposure to light, as well as the effects of exogenous substrate

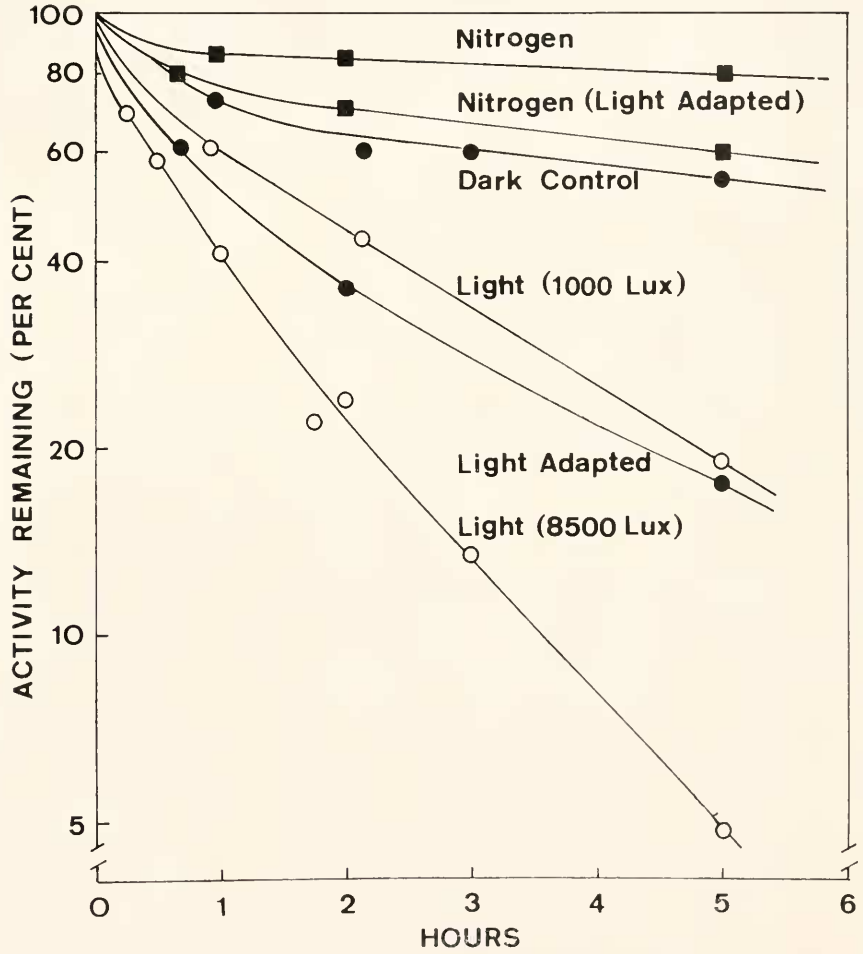

FIGURE 3. Loss of intracellular Na^{22} from *Gracilaria*. Effects of light (8500 lux to 1000 lux) (○), darkness (●), previous exposure to light (48 hours at 8500 lux), anaerobiosis (■), and anaerobiosis after previous exposure to light. Each point represents a measurement of 30–40 branches.

(sodium glutamate, 50 *mM*), were studied in some experiments. Unless otherwise indicated, tissues were adapted for 36–48 hours in either the light or dark, depending upon the subsequent experimental conditions. This conditioning was important since the effect of light on ion movements persisted during 10–20 hours of subsequent darkness.

Average fluxes and rate coefficients were calculated as described, and are listed in Tables II and III. Figures 1–4 show graphically the movements of labeled ions under various environmental conditions. The exchange of extra-

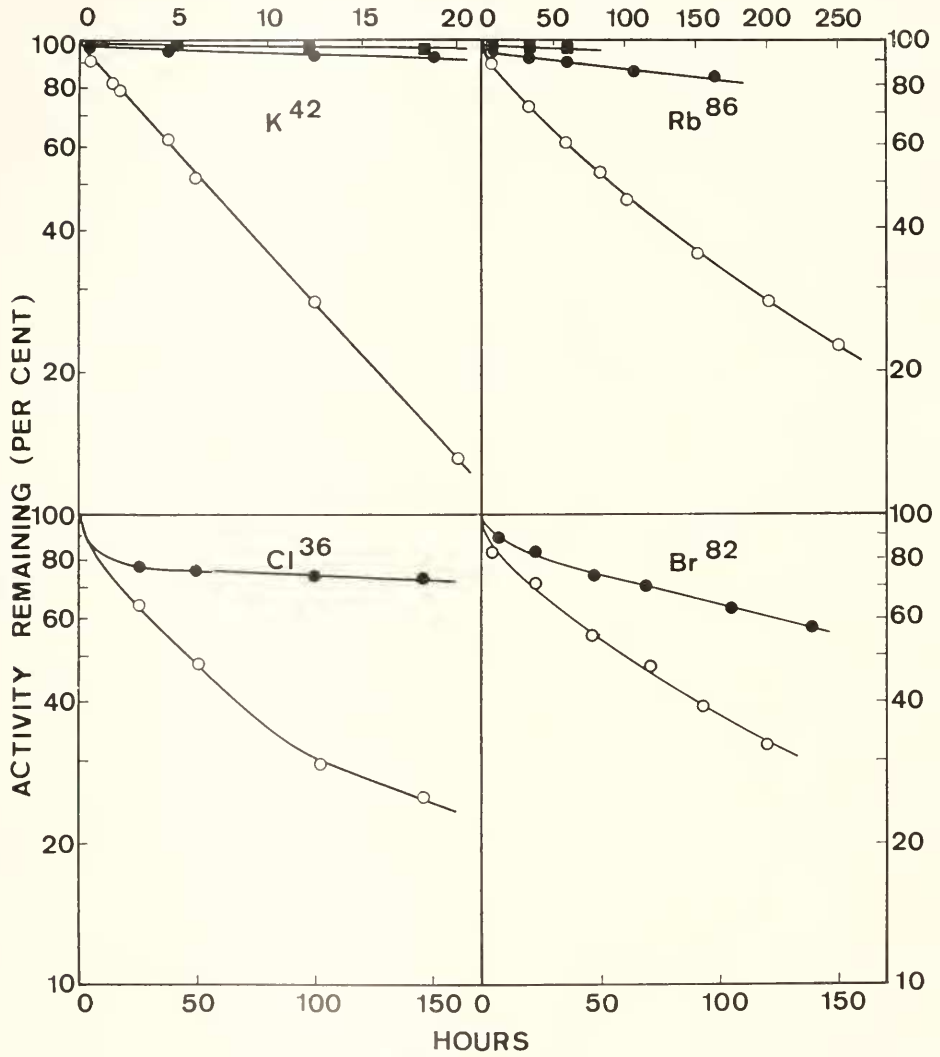

FIGURE 4. Loss of intracellular K^{42}, Rb^{86}, Cl^{36} and Br^{82} from *Gracilaria*. Effects of light (○), darkness (●), and anaerobiosis (■). Each point represents a measurement of 15–40 branches. Note the differences in time scales.

cellular ions is not included in either the figures or the tables, since the tissues were washed before counting. Nevertheless, a small and relatively fast intracellular compartment, or "shoulder," was often evident in both isotope uptake and loss experiments (see Figs. 1–4). This fast compartment also was excluded from the calculations of ion fluxes and rate coefficients. This compartment was generally less sensitive to environmental conditions than was the large slower compartment (presumably the vacuoles of the large cells).

Light promoted both influx and efflux of cations, as well as the efflux of anions (Figs. 1–4, Tables II and III). The effect of light on anion influx was variable, *i.e.*, Cl^- uptake was promoted by light, Br^- uptake was only slightly affected, and I^- uptake was depressed (Fig. 2). Anaerobiosis depressed the influx and efflux of cations and the influx of anions by factors ranging from 0.1 to 0.8, compared to the dark controls (Figs. 1–4).

Previous exposure to light had a temporary stimulating effect on K^+ influx and Na^+ efflux (Figs. 1 and 3). However, light-adapted cells placed in a radioactive environment under nitrogen did not show the temporary stimulation of the K^+ or Na^+ flux unless air was provided (Figs. 1 and 3). In the dark, glutamate (50 mM) increased the K^+ and Na^+ influx 2–3 times, compared to controls.

In general, the movements of Na^+, K^+, Rb^+ and Cs^+ were affected in a qualitatively similar manner by each of the environmental conditions shown in Figure 1. The major difference was that the rate coefficients for isotope influx and efflux decreased markedly in the order $Na^+ > K^+ > Rb^+, Cs^+$ (Table III).

Discussion

All of the major ions (Na^+, K^+ and Cl^-) appear to be actively transported in *Gracilaria*, as indicated by the large differences between the vacuole potential (−81 mv.) and the equilibrium potentials shown in Table I. Sodium, as in most plant and animal cells, is farthest from electrochemical equilibrium ($E - E_{Na} = -131$ mv.), and is extruded against both electrical and chemical gradients. Active Cl^- absorption is indicated ($E - E_{Cl} = -77$ mv.), which is in contrast to most animal, but not plant, cells (see Dainty, 1962). Potassium, which is usually close to electrochemical equilibrium in both plant and animal cells, appears also to be actively absorbed ($E - E_K = 24$ mv.).

One of the assumptions on which the above interpretations rest was that the influx and efflux of an ion are equal. Although this was approximately true, there were some apparent deviations (Tables II and III). These may be due to net ion uptake with growth, as well as to errors involved in estimating fluxes and rate coefficients in a heterogeneous cell population. An alternative value, however, for the predicted vacuole potential, E_j, can be calculated by using the Ussing-Teorell flux-ratio equation (see Dainty, 1962, p. 384). The values for E_j obtained with this equation make even more stringent the requirements for active transport of most of the ions. That is, the $E - E_j$ values are larger than those obtained using the Nernst equilibrium potentials. The only exceptions were that $E - E_{Cl}$ decreased from −77 to −70, and $E - E_{Br}$ decreased from −67 to −65.

A second assumption was that selective binding of ions does not appreciably alter the estimated intracellular concentrations, especially of K^+ and Rb^+. (Any intracellular binding of Na^+ would make the requirements for active Na^+ extrusion even

more stringent.) This was shown to be unlikely, since killed cells quickly lost all of their accumulated K^+ and Rb^+, and since (in the case of Rb^+) uptake was exactly proportional to external concentration over a wide range of low concentrations.

The third possible error could arise from a large difference between the cytoplasmic and vacuolar ion concentrations or electrical potentials. The main potential drop in plant cells, however, appears to be at the plasmalemma rather than at the tonoplast (see Dainty, 1962; Findlay and Hope, 1964; Spanswick and Williams, 1964). Also, since the vacuole:cytoplasm ratio in *Gracilaria* is high, it is unlikely that moderate differences between the ion concentrations in the cytoplasm and vacuole would appreciably change the calculated $E - E_j$ values for these ions.

Finally, the large differences between the C_i/C_o values for K^+, Rb^+ and Cs^+ (Table I) provide independent evidence for the involvement of an active process in the distribution of these ions. Otherwise the C_i/C_o values for these ions would be similar, regardless of the membrane potential. Once again, the absence of selective intracellular ion binding is an important requirement (see Relman *et al.*, 1957).

The present results may be compared with MacRobbie and Dainty's (1958a) study of Na^+ and K^+ fluxes in the seaweed, *Rhodymenia*. Their findings of an active Na^+ efflux and active K^+ influx were similar to those presented here. One exception was their observation that light had only a small effect on Na^+ fluxes, suggesting that Na^+ transport was more closely related to respiration than to photosynthesis. Their light intensity, however, was about 25% of that used in the present study. As shown in Figure 3, Na^+ efflux decreased by a factor of about 0.65 when the light intensity was reduced from about 8500 lux to about 1000 lux. Also, as shown in Figures 1 and 3, the effects of light on ion movements might be obscured if the cells used for measuring fluxes in the dark were not previously adapted to darkness.

A stimulating effect of light on ion transport in plant cells has been reported often, although the mechanism of light stimulation is not clear (see Briggs, Hope and Robertson, 1961; Dainty, 1964). In *Nitella* it was recently suggested that active K^+ uptake is supported by light energy through the utilization of ATP produced in photophosphorylation, whereas active Cl^- uptake is directly linked to light-driven electron-transfer reactions associated with oxygen evolution in photosynthesis (MacRobbie, 1965). The present observations on *Gracilaria* do little to clarify the way in which light promotes active ion fluxes (primarily Na^+ efflux and K^+ and Cl^- influx). With respect to the cation fluxes, the stimulating effects of previous exposure to light were inhibited by anaerobiosis (Figs. 1 and 3). If these fluxes were ATP-dependent, however, and ATP was synthesized in the light, then one might expect that previous exposure to light would stimulate the fluxes in nitrogen as well as in air.

The variable effect of light on anion uptake was an unexpected finding. Chloride uptake was promoted by light, Br^- uptake was only slightly affected, and I^- uptake was depressed in the light (Fig. 2). In contrast, Br^- uptake by *Nitella* was stimulated by light to a greater extent than was Cl^- uptake (MacRobbie, 1962). The depressing effect of light on I^- uptake was found to be even greater in three other seaweeds, *Ulva lactuca*, *Fucus vesiculosus* and *Hypnea musciformis*, than in *Gracilaria* (unpublished data). This appeared in every case to be due to a larger amount of bound I^{131} in tissues which absorbed I^{131} in the dark. Previous studies

(see Shaw, 1962) suggest that in marine algae most of the intracellular iodine exists as iodide, although it may enter the cell in another form, thus raising doubts about the active transport of iodide, *per se*. Evidence from this and earlier studies, however, indicates that iodine is absorbed by an energy-dependent, if not, strictly speaking, an active transport process.

The passive ion fluxes, mainly Na^+ influx and K^+, Rb^+, Cs^+, Cl^-, Br^-, and I^- efflux, were all stimulated by light (Figs. 2 and 4, Tables II and III). It is possible that light simply increased the passive permeability to the anions, since the rate coefficients for Cl^-, Br^- and I^- efflux were similar and their hydrated ion sizes are similar (see Briggs, Hope and Robertson, 1961). The mechanism of passive cation fluxes, however, may be different, *i.e.*, not simple diffusion. This is because the rate coefficients of efflux were in the order $K^+ > Rb^+ > Cs^+$, which is not consistent with predictions based on size or mobility of the hydrated ions (see Ussing, 1960; Briggs, Hope and Robertson, 1961). Thus an important part of the K^+ efflux in *Gracilaria* probably occurs by a mediated, but not active, process—possibly exchange diffusion. This argument may be extended to include Na^+, since the hydrated Na^+ ion is larger than K^+. A similar suggestion was made by MacRobbie and Dainty (1958a) for *Rhodymenia*, based on calculations of an unusually high energy requirement for supporting active Na^+ efflux. Their suggestion would be applicable also to Na^+ efflux in *Gracilaria*.

The ability of *Gracilaria* to maintain its high intracellular K:Na ratio in the absence of both light and oxygen is in contrast to many higher plant and animal cells, as well as some other marine algae. *Ulva lactuca* and *Fucus vesiculosus,* for example, were unable to maintain their normal high K:Na ratio under anaerobic conditions, losing over 50% of their intracellular K^+ in 24 hours (unpublished data). In *Fucus* the loss of K^+ was irreversible after 48 hours. Interestingly, both these seaweeds are highly resistant to extremes of temperature and dehydration (Kanwisher, 1957). *Porphyra* also has been found to lose K^+ and gain Na^+ when exposed to nitrogen (Eppley, 1958a).

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Summary

1. Distribution and transport of alkali and halide ions were studied in *Gracilaria foliifera*, a red marine alga. Ion concentrations, fluxes and rate coefficients were measured with radioisotopes and standard chemical techniques. The intracellular (vacuole) potential was measured with microcapillary electrodes and a high-impedance voltmeter.

2. Intracellular ion concentrations were estimated after correcting for 89% tissue water and 19% extracellular space. Intracellular concentrations of the major ions were (meq./kg. cell water): Na^+, 66; K^+, 680; Cl^-, 462. All of the

intracellular ions except I^- appeared to be completely exchangeable and were probably in solution in the large cell vacuole.

3. Nernst equilibrium potentials, calculated for each ion under conditions of approximate flux equilibrium, were (mv.): Na^+, +50; K^+, −105; Rb^+, −127; Cs^+, −85; Cl^-, −4; Br^-, −14; and I^-, +128. Comparison of these values with the measured vacuole potential of −81 mv. suggested active extrusion of Na^+ and active uptake of K^+, Rb^+, Cl^- and Br^-. Cesium was in approximate electrochemical equilibrium, and the status of I^- was uncertain due to some intracellular binding.

4. Cation fluxes were stimulated by light and, to a lesser extent, by exogenous substrate (glutamate). Previous exposure to light had a temporary stimulating effect on cation fluxes, provided oxygen was present. Anaerobiosis (nitrogen) depressed both influx and efflux of cations. Rate coefficients for the cation were in the order $Na^+ > K^+ > Rb^+, Cs^+$.

5. Anion fluxes were variably affected by light. Chloride uptake was promoted by light, Br^- uptake was slightly stimulated, and I^- uptake was depressed. Anion efflux, however, was consistently stimulated by light. Anaerobiosis inhibited the uptake of all anions. Rate coefficients for anion fluxes were similar to each other but were generally lower than the rate coefficients for cation fluxes.

6. With the exception of I^-, neither light nor anaerobiosis had much effect on the steady-state distribution of ions in *Gracilaria*. The intracellular I^- concentration was much higher in the dark than in the light. This was also found in several other seaweeds, and appeared to be due to an increased amount of bound I^- in non-photosynthesizing tissues.

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